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Correlations between Enzyme Profiles in Cephalopod Muscle and Swimming Behavior[1]

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ABSTRACT: The maximum activities of octopine dehydrogenase, lactate dehydrogenase, alanopine dehydrogenase, citrate synthetase, α-glycerophosphate dehydrogenase, malate dehydrogenase, and glutamate oxaloacetate transaminase were measured in a range of muscles used in swimming by octopods, squids, cuttlefishes, and a nautiloid. The high activities of octopine dehydrogenase and the positive correlation between the activities of Krebs cycle enzymes and enzymes used in the cytoplasmic reoxidation of NADH during aerobic glycolysis indicate the importance of carbohydrates as a major fuel during both anaerobic and aerobic muscle work. The maximum activities of enzymes associated with anaerobic and aerobic carbohydrate catabolism correlate well with the ways in which cephalopod muscles are used in providing propulsion during swimming.

CEPHALOPODS DISPLAY A WIDE RANGE of swimming behavior, varying both in the muscles used in propulsion and in the intensity and duration of the work performed. For example, in squids, cuttlefishes, and octopods, contractions of the mantle muscle are used in powering both slow and rapid jetting; the arms of octopods are used in rowing; and the funnel and retractor muscles of nautiloids eject water from the mantle cavity. It is difficult to observe these animals in nature displaying their full repertoire of swimming behavior and one is forced to rely on clues provided by morphology, physiology, or biochemistry to derive this information.

In the search for correlations between locomotion and biochemical parameters, studies of the relative contributions of aerobic and anaerobic metabolism in supplying energy for muscle work are helpful. During short-term bursts of maximum activity, oxygen levels in muscle may become limiting. When this occurs, adenosine 5′-triphosphate (ATP) is generated from the breakdown of carbohydrates via glycolysis, with possible contributions from additional substrate-level phosphorylations coupled to amino acid catabolism. Under longer-term submaximal work loads, adequate oxygen levels are maintained and ATP can be obtained from oxidative phosphorylation following the complete oxidation of lipids, carbohydrates, or amino acids (Hochachka and Somero 1973).

One method of estimating the relative contributions of aerobic and anaerobic metabolism during locomotion is to determine the maximum activities of enzymes unique to the different catabolic pathways (Baldwin and Seymour 1977; Baldwin, Friedman, and Lillywhite 1977; Muller and Baldwin 1978; Newsholme and Start 1973). For this technique to be used successfully, it is necessary to understand the metabolic organization of the muscles being studied. While it cannot be claimed that the energy metabolism of cephalopod muscle is fully understood, sufficient information is available to permit selection of enzymes that should indicate the relative contributions of

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aerobic and anaerobic metabolism, both in different muscles from an individual animal and for the same muscles in different species.

Cephalopod muscle metabolism differs from the general vertebrate pattern in at least two important features. First, lipids do not appear to be a significant fuel, and carbohydrates and possibly amino acids are catabolized during both aerobic and anaerobic muscle work (Hochachka, French, and Meredith 1978; Hochachka et al. 1975; Storey and Storey 1978; Storey, Fields, and Hochachka 1978). Second, the final step in anaerobic glycolysis usually is catalyzed by octopine dehydrogenase rather than lactate dehydrogenase, and octopine accumulates as an anaerobic end product (Baldwin and England 1980; Grieshaber and Gäde 1976; Hochachka, Hartline, and Fields 1977; Storey and Storey 1978). Thus, while the activities of Krebs cycle enzymes provide information on the aerobic oxidation of all fuels, rate-limiting glycolytic enzymes such as phosphofructokinase are used during both aerobic and anaerobic conditions, and only the terminal glycolytic reaction is unique to anaerobic metabolism. When carbohydrates are catabolized aerobically, both the α-glycerophosphate and malate–aspartate cycles may be involved in the cytoplasmic reoxidation of glycolytically produced NADH (Hochachka et al. 1975, Zammit and Newsholme 1976).

In this study, the activity of octopine dehydrogenase was used as an index of the use of a muscle for short-term bursts of anaerobic work. Citrate synthetase (Krebs cycle), α-glycerophosphate dehydrogenase (α-glycerophosphate cycle), malate dehydrogenase (Krebs cycle and malate–aspartate cycle), and glutamate oxaloacetate transaminase (malate–aspartate cycle) were chosen as enzymes used during longer-term submaximal aerobic muscle work. Participation in the 1979 R/V *Alpha Helix* expedition to the Republic of the Philippines provided an opportunity to compare the maximum activities of these enzymes in fresh muscle samples from a wide range of cephalopods. The results obtained provide insights into both the energy metabolism of cephalopod muscle and the swimming behavior of the animals sampled.

MATERIALS AND METHODS

Experimental Animals

Live cephalopods obtained from local fishermen in the southern Philippines were held in circulating seawater tanks until sacrificed. Juvenile *Sepioteuthis lessoniana* were hatched in captivity.

Preparation of Muscle Homogenates

Samples of fresh muscle were finely minced with scissors after removal of skin and homogenized in 10 vol of ice-cold 50 mM sodium phosphate buffer, pH 7.0. The homogenate was centrifuged at 600 × *g* for 5 min at 4°C to remove cell debris, and the supernatant was assayed immediately. All assays were completed within 90 min of tissue preparation.

Enzyme Assays

Enzyme activities were measured with a Zeiss DM4 recording spectrophotometer in which the cell temperature was controlled by a circulating water bath. Citrate synthetase was assayed at 412 nm, and the other enzyme reactions were followed at 340 nm. Suitable controls were run to allow for nonspecific activity, and determinations were made at 25°C, which approximates the habitat temperature of many of the animals examined. Assays were carried out with 2–25 μl of muscle extract in a total reaction volume of 1 ml. The compositions of the reaction mixtures, which were selected to give maximum activities, were as follows: (1) octopine dehydrogenase: 5 mM pyruvate, 20 mM arginine, 0.2 mM NADH, 50 mM sodium phosphate buffer, pH 7.0; (2) lactate dehydrogenase: 5 mM pyruvate, 0.2 mM NADH, 50 mM sodium phosphate buffer, pH 7.4; (3) alanopine dehydrogenase: 5 mM pyruvate, 200 mM glycine, 0.2 mM NADH, 50 mM sodium phosphate buffer, pH 7.0; (4) α-glycerophosphate dehydrogenase: 0.5 mM dihydroxyacetone phosphate, 0.2 mM NADH, 50 mM sodium phosphate buffer, pH 7.5; (5) citrate synthetase: 0.5 mM oxaloacetate, 0.1 mM acetyl CoA, 0.2 mM 5,5-dithiobis-(2-nitrobenzoic acid), 50 mM Tris–HCl buffer, pH 8.0; (6) malate dehy-

TABLE 1

MAXIMUM ACTIVITIES OF ENZYMES IN MUSCLES FROM CEPHALOPODS

ANIMAL	MUSCLE	ODH	LDH	AlaDH	CITRATE SYNTHETASE	αGPDH	MDH	GOT	ODH/αGPDH+GOT RATIO
Octopods									
Octopus macropus	(1) Mantle	321	19.1	< 0.2	4.4	6.8	55.4	23.2	10.7
	(1) Arm	251	16.2	< 0.2	0.2	5.1	20.1	7.3	20.2
O. membranaceus	(1) Mantle	447	11.8	< 0.2	3.6	12.3	90.0	20.7	13.6
	(1) Arm	375	6.1	< 0.2	1.1	13.7	24.4	5.6	19.4
O. horridus	(1) Mantle	337	19.1	< 0.2	3.3	12.7	73.0	25.3	8.9
	(1) Arm	339	10.8	< 0.2	1.5	10.9	36.5	11.8	14.9
Squids									
Sepioteuthis lessoniana	(3) Mantle	520 ± 65	4.9 ± 0.5	< 0.2	5.9 ± 1.5	10.3 ± 0.4	59.4 ± 5.2	11.2 ± 0.1	24.2
	(1) Fin	353	2.9	< 0.2	10.0	8.4	111.9	15.6	14.7
Juvenile *S. lessoniana*	(15) Mantle	185	5.3	< 0.2	13.1	6.8	97.3	18.2	7.4
Thysanoteuthis rhombus	(1) Mantle	952	11.6	< 0.2	15.6	4.7	42.3	4.7	101.3
	(1) Fin	391	8.8	< 0.2	62.8	2.0	263.0	50.6	7.4
Symplectoteuthis oualaniensis	(2) Mantle	1,454 ± 47	15.4 ± 2.2	< 0.2	4.9 ± 1.2	34.7 ± 1.4	84.4 ± 12.9	17.1 ± 0.1	28.1
	(2) Fin	648 ± 30	4.1 ± 0.6	< 0.2	10.4 ± 3.4	9.4 ± 4.4	203.0 ± 37.0	50.1 ± 5.8	10.9
Cuttlefishes									
Sepia bandensis	(1) Mantle	251	4.4	< 0.2	2.2	3.3	38.0	8.8	20.7
	(1) Fin	394	5.2	< 0.2	5.7	6.8	148.4	37.2	9.0
S. latimanus	(1) Mantle	646	15.6	< 0.2	3.0	14.8	32.5	9.1	27.0
	(1) Fin	468	8.7	< 0.2	6.4	10.2	97.7	32.3	11.0
Nautiloids									
Nautilus pompilius	(1) Funnel	55.5	2.0	< 0.2	2.3	1.8	69.6	7.1	6.2
	(1) Retractor	87.5	3.7	< 0.2	6.2	6.2	93.4	9.2	5.7

NOTE: Data expressed in micromoles per minute per gram wet weight muscle. Temperature, 25°C. ODH, octopine dehydrogenase; LDH, lactate dehydrogenase; AlaDH, alanopine dehydrogenase; αGPDH, α-glycerophosphate dehydrogenase; MDH, NAD^+-malate dehydrogenase; GOT, glutamate oxaloacetate transaminase. The number of muscles assayed is given in parentheses. Where this number is greater than 1, the data given are the mean ± range, with the exception of juvenile *S. lessoniana* where mantle muscles from 15 individuals were pooled for homogenization because of the small amount of tissue.

drogenase: 0.5 mM oxaloacetate, 0.2 mM NADH, 50 mM Tris–HCl buffer, pH 7.5; (7) glutamate oxaloacetate transaminase: 10 mM α-oxoglutarate, 50 mM aspartate, 0.2 mM NADH, 0.1 mM pyridoxyl phosphate, 5 IU malate dehydrogenase, 50 mM Tris–HCl buffer, pH 7.5.

RESULTS AND DISCUSSION

The maximum activities of octopine dehydrogenase, lactate dehydrogenase, alanopine dehydrogenase, citrate synthetase, α-glycerophosphate dehydrogenase, malate dehydrogena :, and glutamate oxaloacetate transaminase and the ratio of octopine dehydrogenase to α-glycerophosphate dehydrogenase + glutamate oxaloacetate transaminase are listed in Table 1.

Carbohydrates as Fuel in Cephalopod Muscles

If carbohydrates are a major fuel used during aerobic muscle work, then the activities of Krebs cycle enzymes should be positively correlated with the activities of enzymes involved in the aerobic oxidation of glycolytically derived NADH.

In the octopods, higher activities of citrate synthetase, malate dehydrogenase, and glutamate oxaloacetate transaminase occurred in mantle than in arm, while α-glycerophosphate dehydrogenase activities were approximately equal in both muscles. A similar relationship between these enzymes was found in the two cuttlefishes and in the long-finned squid *Sepioteuthis lessoniana*, with higher activities of citrate synthetase, malate dehydrogenase, and glutamate oxaloacetate transaminase in fin than in mantle. The highest activity of citrate synthetase was found associated with the highest activity of malate dehydrogenase and glutamate oxaloacetate transaminase in *Thysanoteuthis rhombus* fin. However, in *Symplectoteuthis oualaniensis*, relatively low activities of citrate synthetase occurred together with high activities of α-glycerophosphate dehydrogenase in the mantle and high activities of glutamate oxaloacetate transaminase and malate dehydrogenase in the fin. The funnel and retractor muscles of *Nautilus* had low activities of citrate synthetase, α-glycerophosphate dehydrogenase, and glutamate oxaloacetate transaminase.

Therefore, with the possible exception of *Symplectoteuthis* (see later discussion), a positive correlation can be demonstrated between the maximum activities of the Krebs cycle enzymes citrate synthetase and malate dehydrogenase, and enzymes involved in regeneration of cytoplasmic NAD^+ during aerobic glycolysis. This correlation is taken as evidence for the importance of carbohydrates as a major aerobic fuel in cephalopod muscles.

Of the three dehydrogenases that could be used for the regeneration of cytoplasmic NAD^+ during anaerobic glycolysis, octopine dehydrogenase was clearly the most important in all muscles examined. Alanopine dehydrogenase, which occurs at high activities in the muscles of some bivalves and gastropods (Baldwin and England, this issue; Fields and Hochachka 1981), was not detected in any of the cephalopod muscles; and lactate dehydrogenase activities ranged from only 1 to 6% of the octopine dehydrogenase values. The very high activities of octopine dehydrogenase in cephalopod muscle highlight the importance of carbohydrates as an anaerobic fuel.

Estimating Dependence on Aerobic and Anaerobic Metabolism from Enzyme Profile Data

The maximum activities of the enzymes assayed in this study should reflect the extent to which the various cephalopod muscles utilize aerobic and anaerobic metabolism during swimming, providing information on the intensity and duration of the work performed. However, unless the enzymes are rate-limiting, their activities will exceed the maximum flux through the pathway, and the information obtained will be largely qualitative.

Comparisons of the maximum activities of octopine dehydrogenase and phosphofructokinase in muscles from a wide range of mollusks show that octopine dehydrogenase is not rate-limiting during anaerobic glycolysis, although high activities of the enzyme are

positively correlated with heavy dependence on short-term bursts of anaerobic muscle work (Baldwin and England, this issue; Baldwin and Opie 1978; Zammit and Newsholme 1976). Similarly, in tissues that use carbohydrates aerobically, such as insect flight muscle and gastropod radular muscle, the very high activities of α-glycerophosphate dehydrogenase and glutamate oxaloacetate transaminase, respectively, are considerably greater than twice the activity of phosphofructokinase (Crabtree and Newsholme 1972, Zammit and Newsholme 1976). The activities of citrate synthetase are highest in muscles capable of high levels of sustained aerobic activity, but it is not known whether the reaction catalyzed by this enzyme limits flux through the Krebs cycle in cephalopod muscle (Alp, Newsholme, and Zammit 1976). Thus, while the enzymes assayed in this study may not be rate-limiting, their maximum activities still correlate well with reliance on aerobic and anaerobic metabolism in muscles from a wide range of animals.

Relationship between Enzyme Activities and Swimming Behavior

In analyzing the enzyme profile data, the maximum activity of octopine dehydrogenase was used as an index of reliance on short-term bursts of very rapid swimming; and the maximum activities of citrate synthetase, α-glycerophosphate dehydrogenase, malate dehydrogenase, and glutamate oxaloacetate transaminase were used as indices of slower, sustained swimming. The ratio of octopine dehydrogenase to α-glycerophosphate dehydrogenase + glutamate oxaloacetate transaminase (anaerobic–aerobic ratio) provided a useful indication of the relative importance of these two swimming styles for a particular muscle.

Octopods

Octopods may use both jetting from the mantle cavity and rowing with the arms to provide propulsion during swimming (Baldwin and England 1980). The enzyme profiles obtained for *Octopus macropus*, *O. membranaceus*, and *O. horridus* were similar. Octopine dehydrogenase activities were essentially the same in mantle and arm, while the activities of citrate synthetase, malate dehydrogenase, and glutamate oxaloacetate transaminase were two- to fourfold greater in the mantle. In each species, the anaerobic–aerobic ratio was lower in the mantle than in the arm.

These results suggest that while both the mantle and arms may be used during short-term bursts of rapid anaerobic swimming, the mantle is also capable of sustained submaximal aerobic work associated with slower swimming and respiratory movements. This interpretation is in good agreement with metabolite changes observed in the blue-ringed octopus *Hapalochlaena maculosa* during swimming (Baldwin and England 1980). When this octopus is exercised to exhaustion, octopine accumulates to similar levels in both mantle and arms. However, in animals that have been freeze-clamped before exhaustion, octopine concentrations rise only in the arms, reflecting the higher aerobic capabilities of the mantle muscle.

Squids

In the long-finned squid *Sepioteuthis lessoniana*, the fin extends down the entire mantle edge and is used continuously in an undulating motion for maintaining position and for sustained slow-speed swimming. The mantle is involved with gentle continuous respiratory movements and short-term bursts of rapid jetting.

The activities of citrate synthetase, malate dehydrogenase, and glutamate oxaloacetate transaminase were higher in fin than in mantle, while higher activities of octopine dehydrogenase occurred in the mantle. In juveniles of this species, the fin is relatively undeveloped, occurring as a small flap of muscle at the posterior edge of the mantle, and the mantle is used for both slow, sustained and rapid-burst swimming. Lower activities of octopine dehydrogenase; higher activities of citrate synthetase, malate dehydrogenase, and glutamate oxaloacetate transaminase; and a lower anaerobic–aerobic ratio in the

mantle of the juvenile, relative to the adult mantle, are in keeping with a shift toward increased use of sustained aerobic work and reduced anaerobic capabilities in the juvenile.

The body shapes of the shorter-finned squids reflect adaptations for high-speed swimming. In *Thysanoteuthis rhombus* and *Symplectoteuthis oualaniensis*, the fin is shorter, thicker, and more muscular, and the mantle is more elongated than in *Sepioteuthis*.

The highest activities of citrate synthetase, malate dehydrogenase, and glutamate oxaloacetate transaminase were found in *Thysanoteuthis* fin, indicating use of this muscle to power sustained rapid aerobic swimming. In contrast, the lower activities of aerobic enzymes, higher octopine dehydrogenase activity, and higher anaerobic–aerobic ratio in the mantle suggest that this muscle is designed for short bursts of very high speed jetting.

The results obtained for *Symplectoteuthis* are more difficult to interpret, because relatively low activities of citrate synthetase were associated with relatively high activities of α-glycerophosphate dehydrogenase and malate dehydrogenase in the mantle, and high activities of glutamate oxaloacetate transaminase and malate dehydrogenase in the fin. The simplest explanation is that citrate synthetase was unstable in the muscle homogenates tested. The α-glycerophosphate cycle rather than the malate–aspartate cycle appears to be more important during aerobic carbohydrate metabolism in the mantle muscle; thus, the high malate dehydrogenase activity in this tissue presumably is associated with elevated Krebs cycle activity. Hochachka et al. (1975) and Fields, Baldwin, and Hochachka (1976) obtained quite different enzyme profile data for mantle muscle of a Hawaiian form of *Symplectoteuthis oualaniensis* (citrate synthetase 15, malate dehydrogenase 240, α-glycerophosphate dehydrogenase 280, glutamate oxaloacetate transaminase 20, octopine dehydrogenase 110; all measurements in micromoles substrate per minute per gram wet weight of muscle, at 25°C). Hochachka et al. (1975: 151) argued that this muscle was "sustained by an obligatory aerobic carbohydrate and amino acid catabolism," and possessed little capacity for bursts of anaerobic work. In marked contrast, the octopine dehydrogenase activity obtained for the mantle muscle of *Symplectoteuthis oualaniensis* in the present study is the highest reported for any mollusk muscle, and presumably is associated with very highly developed anaerobic capabilities. However, the anaerobic–aerobic ratio is still considerably lower than for *Thysanoteuthis* mantle, and may indicate greater reliance on aerobic metabolism for sustained high-speed swimming in *Symplectoteuthis*. These differences in enzyme profiles between the Philippine and Hawaiian forms of *Symplectoteuthis oualaniensis* may reflect a taxonomic problem (Young 1975).

Cuttlefishes

The swimming behavior of the two cuttlefishes studied resembles that of the long-finned squid, with the thin elongated fin being used continuously for slow-speed maneuvering and the mantle for respiratory movements and rapid jetting.

The octopine dehydrogenase activities suggest that both mantle and fins are used during short-term bursts of rapid swimming. Storey and Storey (1978) have reported the accumulation of octopine in mantle muscle of the cuttlefish *Sepia officinalis* following enforced swimming to exhaustion, but octopine levels were not determined in the fin. The higher activities of citrate synthetase, malate dehydrogenase, and glutamate oxaloacetate transaminase, and lower anaerobic–aerobic ratios in the fin are associated with the use of this muscle for sustained submaximal aerobic work during position holding and slow swimming.

Nautiloids

Nautilus, confined within a rigid shell, is the least active of the cephalopods studied. The animal swims by jetting, using contractions of both the funnel and retractor muscles to expel water from the mantle cavity. The mantle muscle, which lines and secretes the shell, is not involved in locomotion (Bidder 1962; Packard, Bone, and Hignette 1980). Limited observations of these animals in their natural

habitat show them to be slow swimmers that feed by foraging in a leisurely fashion near the sea floor (Ward, Greenwald, and Greenwald 1980).

The low activities of the aerobic enzymes in both funnel and retractor muscles suggest low levels of aerobic energy metabolism during slow, sustained swimming, and can be correlated with the lower oxygen demands and less efficient oxygen delivery system of *Nautilus* relative to other cephalopods (Johansen, Redmond, and Bourne 1978; Redmond, Bourne, and Johansen 1978). Octopine dehydrogenase activity is also low in funnel and retractor muscles relative to the other cephalopod muscles examined. Although high concentrations of octopine are known to accumulate in the retractor muscle during removal of the animal from the shell or following electrical stimulation of isolated muscle preparations (Hochachka et al. 1977, 1978), it seems that *Nautilus* does not rely heavily on bursts of anaerobic muscle work during swimming.

CONCLUSIONS

The results obtained in this study underline the importance of carbohydrates as a major fuel during both aerobic and anaerobic muscle work associated with swimming in octopods, squids, cuttlefishes, and nautiloids. In all cephalopod muscles examined, with the notable exception of *Symplectoteuthis oualaniensis* mantle, the malate–aspartate cycle appears to be more important than the α-glycerophosphate cycle for regenerating cytoplasmic NAD^+ during aerobic glycolysis. During anaerobic glycolysis, this function is carried out by octopine dehydrogenase rather than lactate or alanopine dehydrogenases.

The maximum activities of enzymes associated with aerobic and anaerobic carbohydrate catabolism correlate well with the ways in which the muscles of cephalopods are used to provide propulsion during swimming. Such enzyme profile data should be of value in predicting swimming behavior in species for which direct behavioral observations are lacking. Determining the maximum activities of enzymes, rather than metabolite changes following enforced exercise in the laboratory, may also have the important advantage of reflecting more accurately the aerobic and anaerobic capabilities of the animal in its natural habitat. With this technique, no assumptions need be made about the relevance of an artificial exercise regime to normal swimming behavior.

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